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The Effect of Ambient pH Variations on the Solubility of Calcium Silicate Based Root Canal Sealers: An In Vitro Study

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Abstract

Background: This study aims to evaluate the solubility of calcium silicate-based root canal sealers (CSBS) under neutral, alkaline, and acidic conditions. These materials are widely recommended in endodontics due to their biocompatibility and bioactivity. However, their hydraulic setting reaction and ion releasing nature make them susceptible to ambient pH fluctuations such as those that may occur in peri radicular tissues during inflammation.

Objective: To evaluate and compare the solubility of two types of calcium silicate-based root canal sealers (BioRoot RCS and TotalFill BC Sealer) against an epoxy resin-based sealer (AH Plus) after immersion in buffered solutions with neutral (pH 7.4), alkaline (pH 9.0), and acidic (pH 5.0) pH levels.

Materials and Methods: 72 standardized disc shaped specimens were prepared (8 specimens per sealer for each pH level) using silicone molds (10 mm in diameter and 2.5 mm in height). Following the completion of setting and drying, solubility was calculated as a percentage of the initial mass loss, using a precision balance (with an accuracy of 0.0001 g). The samples were immersed in 30 mL of various solutions acetate buffer (pH 5.0), phosphate buffered saline (pH 7.4), or Tris HCl buffer (pH 9.0) at 37°C for durations of 24 hours and 7 days. After the samples were redried, their final dry mass (M2) was measured, and the percentage of solubility (S%) was calculated. Statistical analyses were performed using two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test ($p < 0.05$).

Results: An acidic environment led to a significant increase in the solubility of calcium silicate-based sealers (CSBS) compared to neutral or alkaline media ($p < 0.05$), whereas the material "AH Plus" exhibited the lowest solubility rate across all tested pH conditions.

Conclusion: Under the conditions of this study, an acidic environment significantly accelerates the dissolution of calcium silicate-based sealers. Therefore, the inflammatory status of the periapical region should be taken into account when selecting hydraulic sealing materials, in order to ensure the integrity of the apical seal over the long term.

Keywords: Hydrogen ion concentration; calcium silicate; root canal treatment, solubility; root canal filling materials

1. Introduction

Calcium silicate based root canal filling materials are widely recognized for their bioactivity, antimicrobial properties, and ability to set effectively in moist environments. However, concerns persist regarding potential setting failures [1–3] and high solubility [3–5]. Such issues may arise from fluctuations in pH [6], interactions with intra canal medicaments [7], or moisture imbalances [8] factors that can hinder the hydration process and negatively impact mechanical properties. Ultimately, these limitations may compromise the sealing ability and overall clinical performance of the material [9].

Achieving a tight, three dimensional seal of the root canal system is essential for preventing microbial recontamination and promoting the healing of periapical tissues [10]. Root canal sealers are critical for filling anatomical irregularities and gaps at the interface between the core filling material and the root dentin [11]. Among the required physical and chemical properties, low solubility is a primary concern; excessive solubility leads to the formation of voids and gaps at the interfaces, which weakens the apical seal and facilitates microbial microleakage [12]. According to ANSI/ADA and ISO 6876/2012 standards, solubility is evaluated by measuring mass loss after 24 hours of immersion in distilled water; the acceptable limit is 3%, with no visible fragmentation of the original mass [13].

Laboratory factors such as inflammation and bacterial byproducts (mimicking clinical environments) can lower the pH level. This increased acidity may negatively affect the performance of root canal sealers by enhancing their solubility, thereby compromising their sealing ability and, ultimately, their longterm stability [14–16]. Given their direct interaction with living tissues and biological fluids, it is crucial to assess the impact of an acidic environment on root canal filling materials [17].

Over the past decade, calcium silicate based sealers (CSBS) also known as "bioceramic" sealers have gained widespread use in clinical practice. This is due to their antimicrobial properties, alkaline pH, superior biocompatibility, and ability to stimulate the formation of hydroxyapatite at the interface between the sealer and the root dentin [18-19]. Unlike traditional epoxy resin based materials (such as AH Plus), which harden through a polymerization process and exhibit very low solubility, calcium silicate based materials rely on a hydration reaction that produces hydrated calcium silicate gel (CSH) and soluble calcium hydroxide ($Ca(OH)_2$) [20]. Although the release of calcium ions (Ca^{2+}) and hydroxyl ions (OH^-) is what confers bioactivity to these materials, it also renders them inherently more susceptible to mass loss when exposed to aqueous environments [21].

Although many studies have investigated the solubility of root canal sealers under various immersion conditions and at different pH levels, the available evidence is difficult to interpret, as the effects of pH often overlap with the chemical composition of the immersion medium. The use of calcium hydroxide as an intra canal medicament can shift the surrounding microenvironment toward an alkaline state (with a pH ranging from 8.5 to 9.5) [22]. Previous studies have shown that acidic environments interfere with the hydration kinetics of tricalcium silicate and dicalcium silicate, leading to calcium leaching and the degradation of the material's microstructure [23, 14]. Research on the impact of low pH on the solubility of calcium silicate based root canal sealers remains limited. Therefore, this study aimed to evaluate the feasibility of an experimental model for assessing the effect of pH variations in the surrounding environment (5.0, 7.4, and 9.0) on the solubility of two types of calcium silicate based root canal sealers, in comparison to an epoxy resin based root canal sealer. The null hypothesis stated that neither the type of material used nor the pH level of the surrounding environment has an effect on solubility.

2. Materials and Methods

2.1. Preparation of Buffered Immersion Solutions

Three fresh standard buffer solutions were prepared using analytical grade chemicals and deionized water to maintain a constant pH value for the medium throughout the immersion periods:

1. Neutral physiological medium (pH 7.4; Phosphate Buffered Saline, PBS): 6.5 g of sodium chloride (NaCl), 0.15 g of potassium chloride (KCl), 1.44 g

of disodium hydrogen phosphate (Na2HPO4), and 0.20 g of potassium dihydrogen phosphate (KH2PO4) were dissolved in 600 mL of deionized water. The pH was adjusted to 7.40 ± 0.02, and the final volume was brought to 800 mL.

2. Alkaline medium (pH 9.0; 0.1 M TrisHCl buffer): 10.11 g of Tris base was dissolved in 600 mL of deionized water. The pH was adjusted to 9.00 ± 0.02 using 0.8 M hydrochloric acid (HCl), and the final volume was brought to 800 mL.
3. Acidic medium (pH 5.0, 0.1 M acetate buffer solution): 5.77 g of anhydrous sodium acetate (CH3COONa) was dissolved in 600 mL of deionized water, and 1.1 mL of acetic acid (CH3COOH) was added. The pH was then adjusted to 5.0 ± 0.02, and the final volume of the solution was brought to 800 mL.

All solutions were verified using a calibrated digital pH meter before the immersion process began.

2.2. Sample Preparation and Grouping

Three types of root canal sealers were evaluated:

- Group 1:(Control Group): AH Plus (Dentsply DeTrey, Konstanz, Germany) an epoxy resin based sealer. AH Plus Jet was dispensed into the molds using a mixing tip attached to the syringe.
- Group 2: TotalFill BC Sealer (FKG Dentaire, La Chaux de Fonds, Switzerland) a calcium silicate based sealer (ready to use/premixed). The BC Sealer was dispensed directly into the molds using plastic application tips attached to its respective syringe.
- Group 3: BioRoot RCS (Septodont, Saint Maurdes Fossés, France) a calcium silicate based sealer (prepared by mixing a powder with a liquid). BioRoot RCS was placed into the molds using a resin spatula.

To facilitate the setting reaction, 4 µl of distilled water was added to each mold, in accordance with ISO 6876:2012 guidelines.

24 standard disc shaped specimens were prepared for each sealer material (n=8 per subgroup based on pH level; 72 specimens in total). This total sample size was determined to be optimal for detecting statistically significant differences. Silicone molds (10 mm internal diameter, 2.5 mm height; as per ISO 6876 standard dimensions) Figure (1) were used. A thin nylon thread (0.3 mm thick, 10 cm long) was embedded into the paste before it set, allowing the specimens to be suspended vertically. The molds were placed between two glass plates, with layers of nylon placed between the plates and the molds. After filling the molds, the specimens were then kept in an incubator at a temperature of 37±1°C and a relative humidity of 95% for a duration three times the setting time specified by the manufacturer (approximately 24 hours for "AH Plus Jet," and 4 hours each for "BioRoot RCS" and "TotalFill BC Sealer"). A piece of moist gauze was placed inside the incubator to provide the humidity required for the hydraulic setting process. Following the incubation period, the specimens were removed, and any surface protrusions or irregularities were trimmed using a scalpel. Each specimen was then weighed three times using a sensitive electronic balance to determine the mean initial mass.

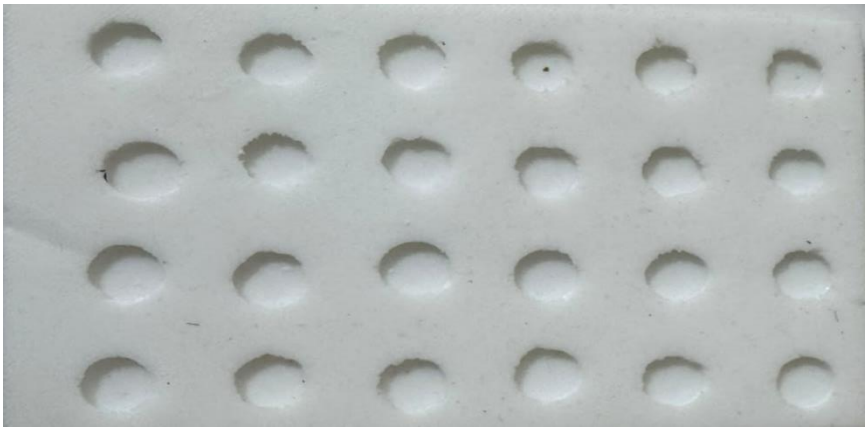


Figure (1) Standardized silicone mold

2.3. Solubility Testing Procedure

After being removed from the molds, the solidified tablets were dried in a desiccator containing freshly dried silica gel at 37°C for 24 hours, until a constant weight was achieved.

Three measurements were taken for each sample using a sensitive analytical balance, and the mean initial dry mass was recorded as M1.

Each tablet was placed individually into a sealed glass tube containing 30 mL of the appropriate buffer solution (pH 5.0, 7.4, or 9.0). The tubes were then kept in an incubator at 37 ± 1°C for 24 hours (and also for 7 days, with the buffer solution replaced daily to prevent any change in pH). Following the immersion period, the samples were rinsed with 2 mL of deionized water, gently blotted dry using filter paper, and returned to the desiccator at 37°C for 24 hours until a constant dry mass was regained. The mean final dry mass was recorded as M2. The percentage of solubility (S%) was calculated as follows:

$$S(\%) = [(M1 - M2) / M1] \times 100$$

2.4. Statistical Analysis

Data were analyzed using SPSS software, version 26.0 (IBM Corporation, Armonk, NY, USA). After verifying the normal distribution of the data using the Shapiro–Wilk test, a two-way ANOVA was performed, followed by Tukey's posthoc tests, to evaluate the effects of sealer type and the pH of the surrounding medium on solubility levels (P = 0.05).

3. Results

The mean solubility percentages and standard deviations for all tested root canal sealers across the three pH environments (pH 5.0, pH 7.4, and pH 9.0) after (1 day and 7 days) of immersion are presented in Tables 1 and 2 and illustrated in Figure 2 (A–C).

Two-way ANOVA revealed statistically significant effects for both the type of sealer ($p < 0.001$) and the pH of the immersion medium ($p < 0.001$). Across all pH levels and both evaluation periods, AH Plus recorded the lowest solubility rate ($p < 0.05$), with no statistically significant differences observed between the acidic, neutral, and alkaline media ($p > 0.05$). TotalFill BC Sealer demonstrated significantly lower solubility compared to BioRoot RCS ($p < 0.05$), while BioRoot RCS exhibited the highest solubility values, particularly in the acidic medium (Figure 2 A–C). Furthermore, immersion in an acidic medium (pH 5.0) resulted in a significant increase in the solubility of both calcium silicate-based materials compared to neutral (pH 7.4) and alkaline (pH 9.0) media ($p < 0.05$), thereby exceeding the 3.0% maximum limit permitted under the international standard ISO 6876:2012.

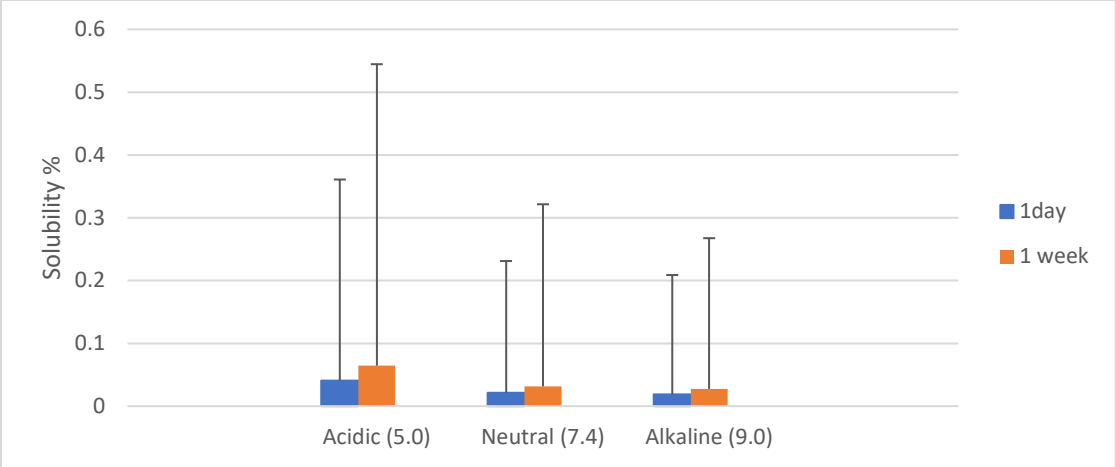
Table 1: Values Mean± SD of Solubility Percentages (S%) After 1 Day of Immersion.

Root Canal Sealer	Acidic (pH 5.0)	Neutral (pH 7.4)	Alkaline (pH 9.0)	p-value (pH Effect)
BioRoot RCS	4.08 ± 0.29%	2.2 ± 0.18%	1.91 ± 0.16%	p < 0.001
TotalFill BC Sealer	3.35 ± 0.24%	1.6± 0.15%	1.43± 0.11%	p < 0.001
AH Plus (Control)	0.22± 0.04%	0.24± 0.04%	0.29± 0.05%	p = 0.139
p-value (Sealer Effect)	p < 0.001	p < 0.001	p < 0.001	—

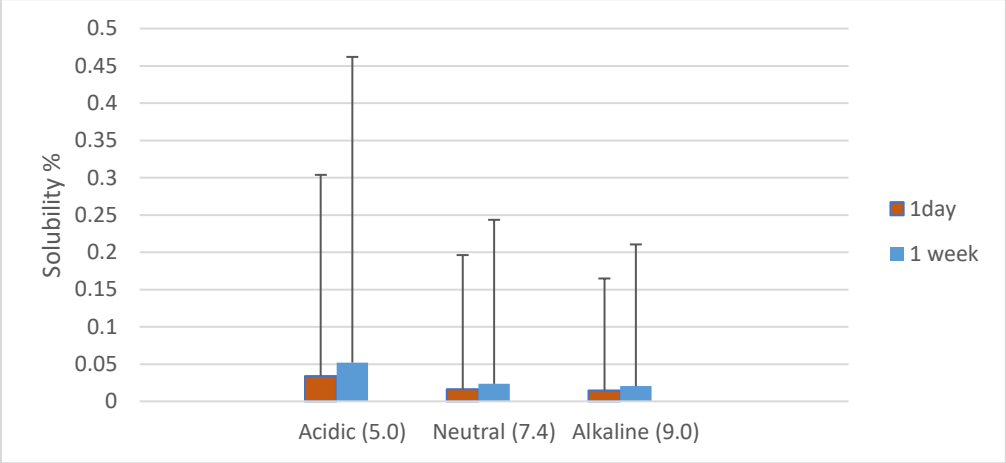
Table 2: Values Mean± SD of Solubility Percentages (S%) After 7Days of Immersion.

Root Canal Sealer	Acidic (pH 5.0)	Neutral (pH 7.4)	Alkaline (pH 9.0)	p-value (pH Effect)
BioRoot RCS	6.40 ± 0.45%	3.11 ± 0.27%	2.70 ± 0.2%	p < 0.001
TotalFill BC Sealer	5.10 ± 0.38%	2.29± 0.19%	2.01± 0.16%	p < 0.001

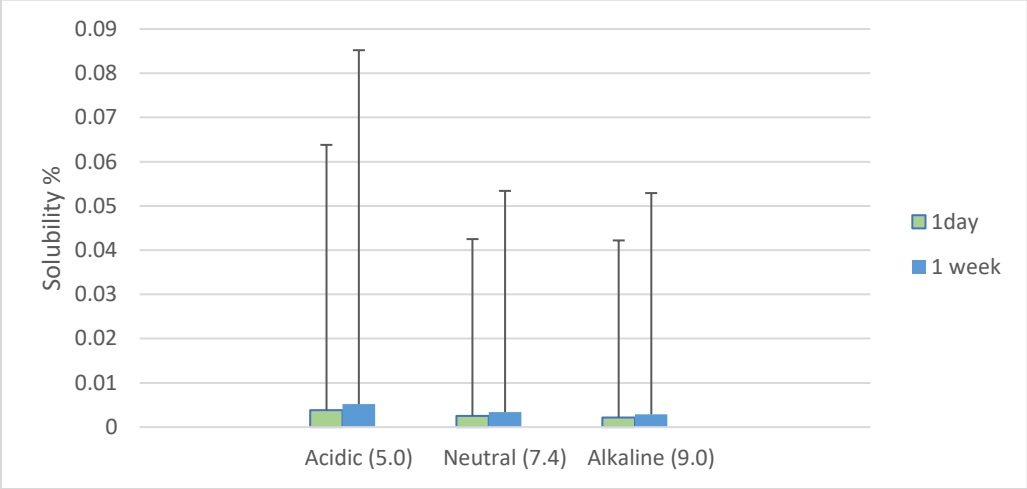
AH Plus (Control)	0.49± 0.07%	0.31± 0.04%	0.27± 0.04%	p = 0.079
p-value (Sealer Effect)	p < 0.001	p < 0.001	p < 0.001	—



(Figure 2 A) BioRoot RCS



(Figure 2 B) TotalFill BC Sealer



(Figure 2 C) AH Plus (Control)

4.Discussion

The dimensional and chemical stability of a root canal sealer within the peri-radicular environment is fundamental to the long term clinical success of root canal obturation, as this environment is inherently dynamic [10-11]. In this study, variations in the pH of the surrounding environment significantly influenced the dissolution behavior of calcium silicate based sealers, leading to the rejection of the null hypothesis.

Across all tested media, AH Plus (an epoxy resin-based sealer) exhibited the lowest dissolution values and excellent stability, consistently remaining well below the 3.0% maximum limit stipulated by the international standard ISO 6876:2012 [13]. This high resistance to dissolution is attributed to strong, hydrophobic covalent bonds that are chemically stable against hydrolysis in aqueous or mildly acidic environments [11, 15]. Furthermore, its structure a cross linked amine epoxy polymer network is a desirable characteristic for root canal sealers, as it helps prevent micro leakage over time. Meanwhile, TotalFill BC Sealer showed moderate solubility. According to ISO standards, solubility levels below 3% are considered acceptable; however, higher solubility was observed for BioRoot RCS in the acidic medium. Overall, all tested materials complied with ISO 6876:2012 limits under neutral and alkaline conditions at 24 hours, whereas acidic conditions pushed both calcium silicate based sealers beyond the 3.0% threshold.

When comparing calcium silicate-based root canal sealers (such as TotalFill BC Sealer and BioRoot RCS) with AH Plus, higher dissolution rates are observed, particularly in acidic environments (pH 5.0). During the hydration of dicalcium and tricalcium silicates, calcium hydroxide ($Ca(OH)_2$) is formed as a soluble by product [20-21]. In neutral (pH 7.4) and alkaline (pH 9.0) environments, the loss of hydroxide (OH^-) and calcium (Ca^{2+}) ions is partially offset by the precipitation of calcium carbonate or calcium phosphate (apatite-like) layers on the surface of the sealer, which limits further mass loss [16,19]. In a phosphate-buffered saline solution (pH 7.4), the interaction between the released calcium ions and the phosphate ions present in the surrounding medium promotes surface biomineralization, which in turn reduces overall mass loss [17]. There are notable differences between various calcium silicate-based root canal sealers such as "BioRoot RCS," which is prepared by mixing a powder with a liquid, and "TotalFill BC Sealer," which comes ready to use. These differences can be attributed to variations in their chemical composition, the agents used to provide radiopacity, the nature of the liquid component in the formulation (e.g., zirconium oxide versus tantalum pentoxide/zirconium oxide), and the water-to-powder ratios [21, 24]. Manually prepared formulations may contain microscopic air voids formed during the mixing process; this increases the surface area exposed to the surrounding environment compared to industrially manufactured, ready-to-use (injectable) pastes [24]. From a clinical perspective, our findings underscore the importance of controlling periapical inflammation and ensuring thorough disinfection of the root canal system before proceeding with obturation using hydraulic calcium silicate-based materials [22, 25].

This results agree with Claudio et.al showed that an epoxy resin-based root canal sealer (AH Plus Jet) meets the solubility standards set by ISO 6876:2012, whereas all the calcium silicate-based sealers tested exceed the acceptable limits. The study also reveals that an acidic environment (pH 5) significantly increases the solubility of calcium silicate-based sealers compared to a neutral environment (pH 7); among these, BioRoot RCS exhibited the lowest solubility, while AH Plus Bioceramic disintegrated after 7 days.

While Anastasios et.al disagree with the result of this study by showing calcium silicate was significantly lower than the internationally permitted maximum (3%) as per the ISO 6876 standard—a finding that contrasts with the higher values recorded in your table (4.08% for BioRoot RCS and 3.35% for TotalFill BC in an acidic environment). The study attributed this to the precipitation of apatite-like crystals on the surface, which enhances the seal and prevents dissolution.

A limitation of this laboratory study is that the samples prepared in the form of discs were completely immersed in static buffer solutions. This results in a significantly larger surface area being exposed than what occurs clinically within a root canal, where the sealing material is confined between the dentin walls and the gutta percha.

Future research conducted on extracted teeth using volumetric analysis via micro computed tomography (micro-CT) under conditions characterized by dynamic pH fluctuations is recommended to validate these findings.

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